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Case report

Immunohistochemical detection of early myocardial damage in two sudden deaths due to intentional butane inhalation. Two case reports with review of literature

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ABSTRACT

The abuse of household and other commercially available products containing volatile organic solvents is underrecognized. Not infrequently intentional butane inhalation results in high morbidity and mortality. A fatal outcome of butane abuse can be caused by asphyxia, cardiac arrhythmia or trauma. The reported number of cases in which death was the consequence of pure butane inhalation is limited, and in most cases a mixture of propellants was involved. This report covers two cases of sudden death due to the sniffing of a cigarette lighter refill containing butane. Autopsy was followed by toxicological, pathohistological and immunohistochemical analysis. Butane gas was confirmed in samples of blood, urine, brain and lungs by the gas chromatography method – "headspace" technique. Histology showed almost identical changes in the lungs and heart in both cases. The morphology of heart damage on standard H/E stains was of special interest because it displayed all the characteristics of chronic and acute myocardial hypoxia found in the absence of atherosclerotic heart disease. In order to confirm early cardiac death caused by asphyxia due to butane inhalation a panel of immunohistochemical agents was used: Myoglobin, Desmin, Fibronectin, Fibrinogen and CC9.

1. Introduction

The intentional inhalation of a volatile substance is an underrecognized form of substance abuse in children and adolescents.[1] Inhalant abuse involves inhaling in a substance directly from its container ("sniffing" or "snorting"), placing a rag soaked in the substance over the nose and mouth and inhaling ("huffing"), or pouring the substance into a plastic bag and breathing the fumes ("bagging").[2] Although experimentation with products containing aliphatic and aromatic hydrocarbons (including glues, gasoline, paints, adhesives, varnishes, paint removers) and halogenated hydrocarbons (such as dry cleaning agents, spray paints, nail polish removers, typewriter correction fluids, aerosolized foods and propellants) among younger adolescents is a common practice seen globally in the last decades, it has been recognized since the early 1900s in Western countries.[3] Inhalants that are commonly abused in order to get "high" contain substances like toluene, butane, propane, fluorocarbons, chlorinated hydrocarbons or acetone.[2] Lethal intoxication is more accidental than suicidal by causing death through several mechanisms: suffocation, trauma after dangerous behavior, vagal inhibition, respiratory depression and "sudden-sniffing death syndrome" following cardiac arrhythmia[4–8] which becomes the cause of death in as many as 50 percent of inhalant-related deaths.[4] The release of a burst of catecholamines that can trigger ventricular fibrillation at the start of inhalant abuse, according to some authors, causes the sudden sniffing death syndrome.[1]

Butane – $CH_3(CH2)_2CH_3$ (synonyms: n-butane, butyl hydride, methylethyl-methane), a liquefied petroleum gas with LC_{50} of 658 g m^{-3}, belongs to a class of aliphatic hydrocarbons which is commonly found in lighter fluid, fuel, spray paint, hair spray, room freshener, deodorants. Frostbite due to rapid cooling on evaporation occurs if butane is used in a liquid state, but most commonly it is abused as a gaseous propellant in an inhalant and acts as a simple asphyxiant causing toxicity by displacing oxygen and preventing it from reaching important tissues and organs.[9]

Our report covers two cases of sudden death occurring after the sniffing of a cigarette lighter refill containing only butane. Most

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reports regard accidental inhalation of a mixture of n-propane, n-butane and isobutane, while only five reported fatal outcomes caused by the intentional inhalation of either n-butane or isobutane.[1,8,10] Histological, immunohistochemical as well as toxicological analyses were performed.

2. Case 1

The dead body of a 25-year-old male, 181 cm high, appropriately nourished, was found lying supine in an abandoned restaurant. A tin lighter refill was found next to the body. On external examination the coroner found no injuries, and ascertained an already well-pronounced rigor mortis and post mortem lividity.

3. Case 2

The dead body of a 14-year-old male, 167 cm high, appropriately nourished, was found by the medical emergency team lying supine in a park after an anonymous tip. A tin lighter refill container was found next to the body (Fig. 1). CPR was attempted with no success. On examination the coroner detected an abrasion frontally right. Rigor mortis and post mortem lividity were not yet pronounced.

Fig. 1. Lighter refill found beside the dead body of a 14-year-old male in case 2.

4. Methods

4.1. Autopsy

Both autopsies were performed in the Forensic Medicine Institute of the Medical School in Zagreb within 24 h of the ascertained time of death. In either case autopsy did not disclose any pre-existing pathological substrate as a possible cause of death, and suspicion regarding the involvement of a third person was not confirmed. Therefore, tissue, biological fluids, gastric contents, nose swab and pubic hair samples were taken for extensive chemical–toxicological analysis:

1) in order to determine the blood and urine level of ethyl alcohol;
2) blood, urine, bile, liver, kidney, gastric content, nose swab and pubic hair samples were analyzed toxicologically for the possible presence of drugs, poison or opiates;
3) because of specific death-related circumstances, that is, of the butane lighter refills found on police investigation next to the bodies, and considering the absence of macroscopic elements which could be used to establish a cause-and-effect link with death, the butane refills were analyzed, and biological brain, lung, blood and urine samples taken.

4.2. Chemical–toxicological analysis

Blood and urine samples, bile liquid, and liver and kidney tissue were analyzed by the extraction technique on an XAD-2 resin column. The nose swab, the pubic hair sample and the gastric content were extracted with organic solvents.

Headspace gas chromatography was used was used to analyze blood, urine (there was no urine in case 2), brain and lung samples. The analysis was performed on a PE AutoSystem Gas Chromatograph with a PE TurboMatrix 40 Headspace Sampler, under the following conditions: column 15%; Hallcomid M-18; O1/Chromosorb; W-HP 80/100 Mesch; column temperature 75 °C; injector temperature 120 °C; detector temperature 130 °C; vial temperature 60 °C; transferline temperature 90 °C; carrier gas N2: 4 ml/min. Sample preparation; 2 ml of t-butanol was placed in a penicillin vial and butane from the lighter refill found by the dead body was added. The vial was close and placed into the headspace sampler and thermostatted at 60 °C for 16 min. Blood, urine, and brain and lung tissue samples obtained from the deceased were prepared in the same way. After thermostatting the sample gas phase was dosed into a PE gas chromatograph, and the obtained chromatograms were compared with the chromatogram of the unquestionable butane sample found by the body.

4.3. Pathohistological analysis

Tissue samples were fixed in 15% formalin, embedded in paraffine, sliced to 5 μm and stained by the H/E method.

4.4. Immunohistochemical analysis

Heart samples were stained by a panel of immunohistochemical agents: Desmin (Dako, 1:50), Myoglobin (Dako, 1:25), Fibrinogen (Dako, 1:40), Fibronectin (Dako, 1:200), CC9 (Novocastra, 1:25). The intensity and distribution of the staining reactions were scored semiquantitatively (Table 1).

Table 1
Immunohistochemical analysis of cardiac damage regarding the earliest positive myocardial tissue reaction.<!?show [accolade4?>

Antigens	Time of survival needed for antigens to react	Type of antigen reaction	Results of semiquantitative analysis	
			Case 1	Case 2
Desmin*	A few minutes	"negative markers" depletion loss displacement	++	++
Myoglobin*			+	+
Fibrinogen**	Half an hour	"positive markers" intra-sarcolemmal accumulation	+	++
Fibronectin**			++	+
CC9***	An hour and more	"positive markers" cytoplasmic or sometimes membranous	+	+

Key: * 0 − negative, + − weak, ++ − strong loss of reaction.
** 0 − either negative or a few disseminated cells with faint staining, + strongly stained disseminated cells or weakly stained patches, ++ − strong reaction of patches or larger areas.
*** 0 − negative, + − staining of disseminated single cells or few small group of cells, ++ − staining of large areas of infarcted cells.

5. Results

5.1. Autopsy findings

5.1.1. External examination

Dead bodies of male persons of appropriate physical development and nourishment, and sexual features, presenting no signs of violent action by third persons. In case 2, frontally right and the right flank, superficial abrasions 2 and 3 cm, respectively, in diameter.

5.1.2. Internal examination

In both cases organs are in the regular site and of appropriate development. A picture of internal organ congestion dominates in both cases. The brain and lungs present large edemas, pronounced particularly in case 2, which also shows signs of a descending herniation of the cerebellar tonsils into the foramen magnum.

5.2. Chemical–toxicological analysis

On analysis 0.00 g/kg of absolute alcohol were found in the urine and blood of Case 1, while trace presence of caffeine and Diazepam (anxiolytic) were found in extracts of blood samples, urine, bile, liver and kidney tissue, and in gastric content. Nose and pubic hair swabs contained no traces of drugs, medicaments and/or their metabolites. The presence of butane in blood, urine, lung and brain samples was conformed by headspace chromatography Fig. 2a.

Blood samples obtained from Case 2 contained 0.00 g/kg of absolute alcohol, and caffeine in bodily fluids and tissues, and nose and pubic hair swabs; no traces of drugs, medicaments and/or their metabolites were found. Headspace chromatography confirmed the presence of butane in blood, lung and brain samples Fig. 2b).

5.3. Histology

Pathohistological analysis discovered an almost identical morphology in both cases. Lung samples were characterized by diffuse intraalveolar haemorrhagic edema with activation of macrophages and erythrophagia. There were also intraalveolarly located uncolored "blebs" resembling those seen in the lungs of drowned persons. In addition to this, occasional intracapillar and endothelial "blebs" were detected, causing total obstruction of the capillar lumen. Moreover, there was total anemia caused by vesicularization and massive congestion of microcirculation in the septal walls. Heart samples revealed chronic changes such as a diffuse and unexpected, considering age, fibrosis of interstitial spaces and periarterial zones of intramural artery branches. Heart samples in Case 1 showed eosionophilic and granulated myofibriles focally. In Case 2 a narrow belt of subendocardially located coagulation necrosis and contraction band necrosis was found.

5.4. Immunohistochemical analysis

The results of semiquantitative analysis of immunohistochemical heart samples stainings in Case 1 and Case 2 are shown in Table 1. Fresh myocardial fiber necrosis was demonstrated by strong and diffuse intracellular loss of cardiac antigen Desmin but poor loss of Myoglobin in both cases. While Fibronectin showed a strong positive reaction of larger areas in Case 1, only disseminated single cells in Case 2 were strongly stained within the patches of weak staining. Fibrinogen showed an exactly opposite reaction. Interestingly, CC9 was not considered to be positive either, although in Case 1 there was some reaction of large areas of infarcted cells but it was rather weak and membranous suggesting earliest deposition. In Case 2 CC9 stained only disseminated single cells (Figs. 3 and 4).

6. Discussion

It is a paradoxical but well known fact that halogenated hydrocarbons commonly known by their trademark name, Freon, also capable of causing death[4] by induction of cardiac arrhythmias,[3] were regularly found as propellants of aerosol cans deliberately used for getting "high" until the nineteen-seventies when they were replaced by non-halogenated hydrocarbons such as butan-propane, n-butane, isobutane, and isopentane because of the protection of the earth ozone layer.[8] In the following years several reports discussed patients who accidentally[11] or deliberately[10] inhaled butane and survived thanks to intensive medical care or CPR, or died in spite of it.[6,8] The mechanism of death was explained by epinephrine-induced cardiac arrhythmias caused by acute inhalation of high concentrations of aliphatic hydrocarbons considered to be otherwise safe. In the past few decades, according to published reports, inhalation of butane became a fairly frequent cause of death especially in young adolecents[1,6,8] or in elders due to autoerotic abuse.[12]

The precise mechanism of sudden death associated with butane inhalation is yet to be determined. Pfeiffer et al. focused on interactions of acute and chronic myocardial changes, and distinct histology of lungs similar to the typical lungs of drowned persons characterized by vesicular transformation of capillary endothelia and development of obstructive microangiopathy. They noticed a non-specific but intense fibrosis not in accordance with the absence of coronary disease and with young age, no signs of myocarditis, while immunohistochemical analysis confirmed acute ischemia. According to authors, an acute ischemic incident could be due to anemia as a consequence of the mechanical barrier in lung circulation manifested by picturesque "blebs" in the alveolar wall

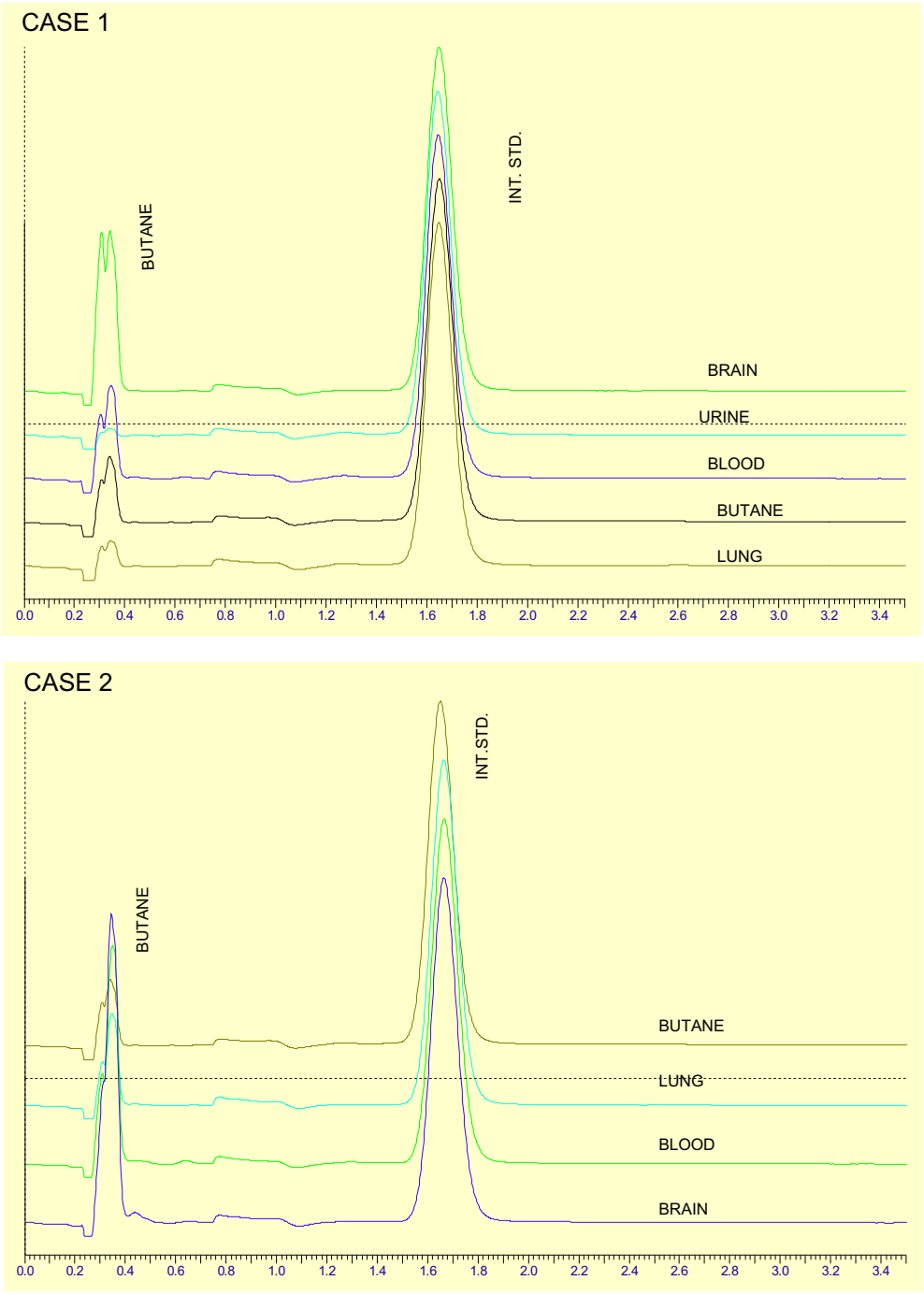

Fig. 2. Gas chromatogram of the tissue samples (a case 1; b case 2).

capillaries. This theory is supported by acute congestion of inner organs found on autopsy.[1]

Sugie et al. emphasize the anesthetic or narcotic effect of n-butane and isobutane on CNS. This happens because of the lipophilic features of butane which is distributed by blood in lipid-rich tissues such as brain and fat-tissues, and also in the liver, heart, and kidney.[13]

Inhalation of hydrocarbons or 0.5–15% butane in the air is suspected to sensitize the myocardium to adrenaline, and the sudden surge of this hormone is thought to result in fatal arrythmia,[1,13] probably ventricular fibrillation, immediately after sudden fear or hard muscular exercise such as fright, running and sexual activity.[8,12,14,15–17]

Summarizing all previously mentioned, hypoxia, respiratory depression and cardiac arrhythmia are probably three possible mechanisms of sudden death related to volatile abuse of inhalants containing butane.

No pathognomonic morphological changes have been detected yet for butane intoxication or sudden death caused by butane or other similar inhalants. Early cardiac death is not only an issue for discussion in forensic medicine, but also a detection problem for the pathologist. Immunohistochemical analysis has improved detection of early ischemic changes in an almost revolutionary manner. Although not used in everyday practice, the possibility of using several immunohistochemical staining for detecting myofibrile

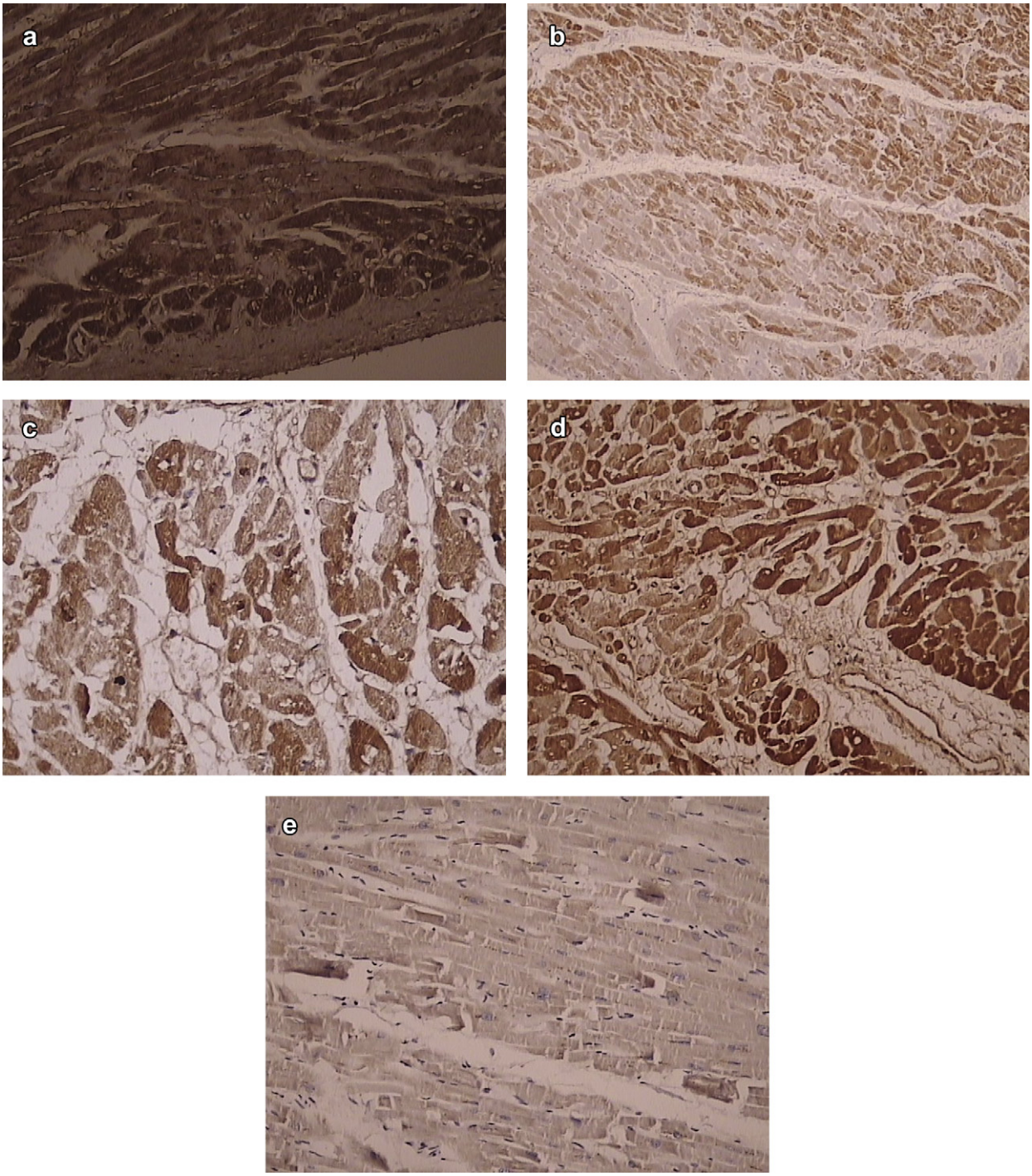

Fig. 3. Immunohistochemical investigation of the myocardium in Case 2 (a Myoglobin: 10×; b Desmin: 20×; c Fibronectin: 10×; d Fibrinogen: 20×; e CC9: 20x).

damage within half an hour of the hypoxic incident is thoroughly presented in several studies[1,16–19] and was even detected in advanced putrefaction.[20] Ortman et al. provided perhaps the best immunohistochemical panel for determining early myocardial damage. They even confirmed that the depletion of cellular antigens (FABP, Troponin C and T, Desmin, Myoglobin, CD 59) begins earlier than the deposition of serum antigens.[17] In fact, it could be detected within minutes after the onset of periods of hypoxia. The deposition of plasma antigens like Fibrinogen and Fibronectin begins earlier than that of C5b-9 which can be visualized 30 min after the onset of AMI. Piercechi et al. even report on complement C9 as a valuable sensitive and specific marker of AMI even more than 1 h after the beginning of myocyte damage.[18] Some authors consider Myoglobin to be even better than FABP and Troponin in the same category of cellular markers.[19] In order to obtain relevant information about whether cardiac death provoked by asphyxia/hypoxia occurred in our cases and after what time after inhalation abuse, we used the immunohistochemical panel shown in Table 1. In that way we covered all the three periods of time recommended by Ortman et al.: cardial death within few minutes, between few minutes and half an hour, and about 1 h after inhalation.[17] Instead of the original complex C5b-9 used in his study the detection of which becomes positive

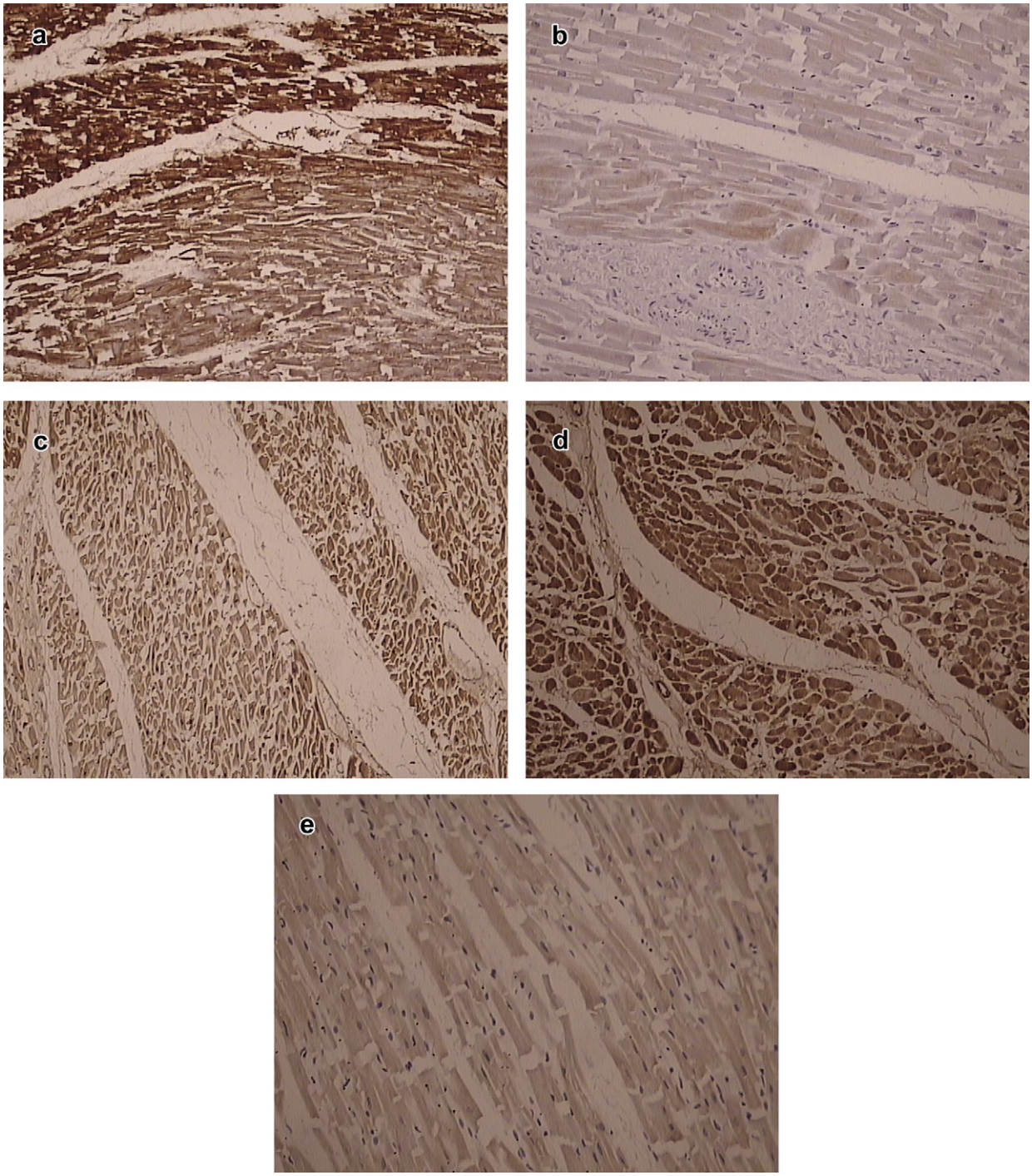

Fig. 4. Immunohistochemical investigation of the myocardium in Case 1 (a Myoglobin: 20×; b Desmin: 10×; c Fibronectin: 40×; d Fibrinogen: 20x; e CC9: 20×).

30–40 min after the onset of ischemia, we decided to use complement C9 in the third group which should be positive 1 h after the hypoxic incident caused by inhalant abuse. Namely, we wanted to separate immediate early cardiac death occurring inside half an hour and delayed early cardiac death occurring one or more hours after the ischemic incident. As reported by Piercecchi-Marti et al., the expression of complement C9 in such an abuse followed by cardiac death has been evaluated to have sensitivity of 85%. Complement C9 deposits are clearly observed as early as 1 h after the ischemic incident. Pierecchi et al. described the appearance of large intense membranous immunostaining in a case with survival of only 30 min suggesting the first phase of the C9 deposit.[18]

Finally, the analysis of biological samples obtained from both cases has shown that they were sober at the time of death and, apart from caffeine and trace Diazepam, no traces of drugs, medicaments or their metabolites were found in either. However, the common feature was the presence of butane in biological blood, lung and brain samples, demonstrating butane abuse.

Although resuscitation was performed in Case 2, no significant decrease of butane concentration was observed. Asphyxia was pointed to be the cause of death in both cases.

All the following findings either confirmed or were at least in accordance with the previously mentioned mechanism of death. Pathohistological analysis focused on samples of lung and heart

samples. Lung sample morphology in both cases was very much like the one seen typically in lungs after drowning.[21] The characteristic vesicular transformation of capillary endothelia could be due to the direct toxic effect of the inhalant. The anemia in the myocardium causing ischemic changes could be a consequence of the mechanical barrier in lung circulation caused by microangiopathy. Pfeiffer et al. also offer another explanation for such a distinct histological picture of the alveolar walls. Namely, because of its lipophilicity, butane could be easily incorporated into the lipid layer of the basal capillary membrane blocking microcirculation.[1] Either way, chronic hypoxia could be the main cause of diffuse chronic changes seen in the myocardium in both cases, especially in Case 1, probably because of older age and longer abuse. Cardiac arrhythmia cannot be excluded as a cause of premortem phenomena, but it cannot be proved either. Therefore, we had to focus on possible early ischemic changes of the myocardium hardly seen in HE stainings but detectable by immunohistochemical agents. Therefore we recommend an immunohistochemical panel (Table 1) helpful for the detection of an acute ischemic cardiac incident/AMI after asphyxiant abuse with no histological features on H/E staining. The results obtained by immunohistochemical analysis of myocardial samples in Case 1 and Case 2, shown in Table 1, were consistent with those reported elsewhere in literature.[1,16,19]

As a positive staining for CC9 Pierchechi et al. considered immunostaining of large areas not disseminated single cells neither a few small piles of cells.[20] Since no strong positive reaction was observed in either of our cases, although in Case 1 a barely seen too weak diffuse reaction could be observed suggesting an initiation of deposition, we excluded early cardiac death or death within 1 h or more. In the same time there was an undoubtedly strong and diffuse reaction of at least one of the negative markers (Myoglobin or/and Desmin) and other positive markers (Fibrinogen or/and Fibronectin) in both cases. Therefore, we concluded the following: first, asphyxia was the main cause of acute myocardial lesion and second, the time of death can be put within a minimum of half an hour, but definitely less than an hour since the abuse. It is clear that butane inhalation caused asphyxia and death, but when we observe the pathophysiological cascade and the histological alveo-septal changes in the lungs which probably contributed to asphyxia or even provoked it, irreparable acute myocardial ischemic lesions were the point with no return.

Conflict of interest

None.

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